Project 1022498

Importance of Mobile Genetic Elements and Conjugal Gene Transfer for Subsurface Microbial Community Adaptation to Biotransformation of Metals

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RESULTS TO DATE: Annual report 2004

Soils used in the present DOE project were obtained from the Field Research Center (FRC) through correspondence with FRC Manager David Watson. We obtained a total of six soils sampled at different distances from the surface: A) Non-contaminated surface soil from Hinds Creek Floodplain (0 mbs (meter below surface). B) Mercury-contaminated surface soil from Lower East Fork Poplar Creek Floodplain (0 mbs). C) Mercury-contaminated subsurface soil from Lower East Fork Poplar Creek Floodplain (0.5 mbs). D) Mercury-contaminated subsurface soil from Lower East Fork Poplar Creek Floodplain (1.0 mbs). E) Non-contaminated surface soil from Ish Creek Floodplain (0 mbs). F) Non-contaminated surface soil from Ish Creek Floodplain (0.5 mbs).

TASK 1. Isolation and characterization of hitherto uncultured bacteria of relevance for biotransformation of metals

Eubacterial 16S rDNA clone libraries have been made. The template DNA was isolated from mercury contaminated and non-contaminated soil sampled 0 m, 0.5 m and 1.0 m from the surface. The clone libraries will be characterized by full length sequencing during the next couple of months in order to investigate the phylogenetic diversity of the soils. The development of a protocol for the isolation of microcolonies (mCFU) directly from filters using a Laser Pressure Catapult microscope has been initiated. Due to methodological difficulties the protocol is still not fully developed but valuable progress has been made. Approaches has been taken to investigate if the fraction of the culturables may increase by incubating filters with mCFU on soil slurry for longer periods on the same slurry or by repeatedly changing the soil slurry. Until now approx. 500 colonies has been isolated whereof most have been grouped by ribosomal spacer PCR.

TASK 2. Horizontal gene transfer to "nonculturable" subsurface bacteria

Horizontal gene transfer optimization experiments combining flow cytometry analysis of gene transfer from E. coli donor cells to indigenous freshwater bacteria have been conducted. The results from these aquatic experiments show that plasmid transfers from donor to indigenous bacteria are much more frequent, than traditional culture-dependent method reveal. The experimental design is working and will now be used for soil microcosms. E. coli donor cells will be used initially, until a Shewanella oneidensis MR-1 has been genetically modified to be used as donor.

TASK 4. Significance of mobile genetic elements for microbial community adaptation to pollutant stress

The mercury concentration has been determined in the contaminated soils. The results showed that the concentrations decreased with depth. In the top soil was found the highest mercury content with 12.47 +/- 1.42 ppm, the middle soil (18?-22?) had a intermediate concentration of 7.59 +/- 0.96 ppm and in the deepest soil (36?-40?) only 1.02 +/- 0.17 ppm. These findings were also reflected in the results of a mercury tolerance assay where the soil was exposed to increasing concentrations of mercury. The experiment was performed in closed serum bottles and every 24 h the development of CO2 was measured. The top soil showed a clear adaptation towards mercury since the respiration only was reduced slightly even at the highest mercury concentrations (100 ppm), whereas the both deeper soils only showed minor adaptation. None of the soils showed any adaptation towards Chromate.

Numbers of bacteria (colony forming units, CFUs) resistant to mercury have been determined in the mercury contaminated and non-contaminated soils mentioned in Task 1. Results showed that the abundance of mercury resistant bacteria was increased in the contaminated soils. Furthermore, adaptation experiments have been performed including diverse microbiological analyses, e.g. plate counts, respiration, and BIOLOG mt2 and EcoMicroPlate readings. In these experiments it was found that the mercury-contaminated soils were more adapted to mercury than the non-contaminated soils. However, pre-exposure of the non-contaminated soils with mercury demonstrated that these soils also had an indigenous mercury resistant population - although at a much lower level than in the contaminated soils.

Initial culture independent detection and quantification of merA, the gene encoding mercuric reductase, conferring bacterial resistance to Hg(II) - by volatilization of Hg2+ to Hg0 - has been done. No merA genes could be detected in any of the soils. However, after pre-exposure to mercury - and elevated populations of mercury resistant bacteria - merA was detected in soil DNA extracted from the contaminated soils.

Microarray work targeting mercury resistance plasmids has been initiated. This work is still in its early stages, with optimizations dominating the work. Plasmids and DNA from the aforementioned soil types will be hybridized to microarrays currently carrying probes for plasmid repA genes and heavy metals resistance genes, including mercury and arsenic. New probes for the microarray slides, targeting the relaxase genes of conjugative plasmids have been designed and will soon be printed on new microarrays.

DELIVERABLES: Oral presentations:

Sorensen SJ 2004 Horizontal Gene Transfer in Natural Environments. Horizontal Gene Flow in Microbial Communities, a DOE/NSF sponsored workshop, June 14-16, Warrenton, VA

Sorensen SJ, Musovic, S, Oregaard G. 2004, Culture independent detection of horizontal gene transfer in natural environments. ISME 10, Cancun, August.

Poster presentations:

de Lipthay JR., Rasmussen LD. & Sorensen SJ. 2004 Introduction of mercury resistant bacterial strains to Hg(II) amended soil microcosms increases the resilience of the natural microbial community to mercury stress. Annual PI-meeting for DOE-NABIR, Washington DC, Marts

Oregaard G., S.J. Sorensen 2004. Single Cell Flow Cytometry Analysis of Gene Transfer from E. coli to Indigenous Freshwater Bacteria Reveals an up to 100 Fold Higher Transfer, than Determined by Selective Culturing. P04: Evolutionary Ecology. ISME 10, August, Cancun